Microspheres of biodegradable polymers were evaluated as a potential controlled-release drug-delivery system in the vitreous. The microspheres were prepared with polymers of poly(lactic acid) or copolymers of glycolic acid and lactic acid. The release of 5-fluorouracil (5-FU) from the microspheres was studied in vitro. Poly(lactic acid) microspheres released 70–85% of total 5-FU over 7 days. Microspheres of polymers with a smaller molecular weight released the drug more rapidly. Copolymer microspheres released 98% of 5-FU over 2 days. The rate of drug release was controllable by changing the molecular weight of the polymers or using a matrix of copolymer. The intravitreal kinetics of the microspheres were studied in ten rabbits in vivo. A suspension of microspheres was injected into the vitreous cavity of five normal eyes and five vitrectomized eyes. By 48 ± 5.2 days after injection, the microspheres disappeared from the vitreous cavity in the five normal eyes. Clearance from the vitreous cavity was accelerated in the five rabbits that underwent vitrectomy (14 ± 2.4 days; P < 0.001). No difference was found in the b waves of electroretinograms before and after injection of the microspheres. The histologic study showed no abnormal findings as a result of the injection. These results suggested that microspheres of biodegradable polymers may be a potential delivery system for the controlled release of drugs in the vitreous. Invest Ophthalmol Vis Sci 32:1785–1790, 1991

Many experimental studies have been done to treat vitreoretinal diseases such as proliferative vitreoretinopathy or endophthalmitis by intravitreal administration of drugs.1–6 However, clinical applications of intravitreal drug therapy have been limited by the relatively short time that it takes for drugs to leave the vitreous cavity.1–3 Repeated injections are required to maintain an effective concentration of the drugs, resulting in an increased incidence of complications. The development of controlled-release drug-delivery systems that can achieve an effective concentration for a specific period has been investigated recently by applying various drug carriers.8,9 Poly(lactic acid) microspheres are one of the promising drug carriers; they are biodegradable in the body.10–12 The microspheres of poly(lactic acid) are resolved into monomers of lactic acid by hydrolytic deesterification before they finally disappear.

The drug 5-fluorouracil (5-FU) has been widely studied as an antiproliferative agent to inhibit cellular proliferation in proliferative vitreoretinopathy1–3 or after glaucoma filtering procedures.13–15 We evaluated, in rabbits, the use of microspheres of biodegradable biopolymers containing 5-FU as a potential controlled-release drug-delivery system in the vitreous.

Materials and Methods
Preparation and Characterization of Microspheres
We prepared the microspheres with two kinds of polymers: poly(lactic acid) and poly(glycolic-lactic acid). The microspheres were prepared by the solvent-evaporation method (Fig. 1). This method was described in detail elsewhere.12 Briefly, 50 mg of 5-FU (Kyowa, Tokyo, Japan) and 450 mg of the polymers were dissolved in 5 ml of N, N-dimethylformamide. The solution was then emulsified in 100 ml of castor oil containing 50 mg of soy bean lecithin by agitation. The oil-in-oil emulsion was agitated for 24 hr under an atmospheric pressure at 45°C until the dimethylformamide solvent was evaporated completely. The solution was then emulsified in 100 ml of castor oil containing 50 mg of soy bean lecithin by agitation. The oil-in-oil emulsion was agitated for 24 hr under an atmospheric pressure at 45°C until the dimethylformamide solvent was evaporated completely. The solution was then emulsified in 100 ml of castor oil containing 50 mg of soy bean lecithin by agitation. The oil-in-oil emulsion was agitated for 24 hr under an atmospheric pressure at 45°C until the dimethylformamide solvent was evaporated completely. The solution was then emulsified in 100 ml of castor oil containing 50 mg of soy bean lecithin by agitation. The oil-in-oil emulsion was agitated for 24 hr under an atmospheric pressure at 45°C until the dimethylformamide solvent was evaporated completely. The solution was then emulsified in 100 ml of castor oil containing 50 mg of soy bean lecithin by agitation. The oil-in-oil emulsion was agitated for 24 hr under an atmospheric pressure at 45°C until the dimethylformamide solvent was evaporated completely.
The size of the microspheres was determined by observation with scanning electron microscopy (SEM, Model S-450; Hitachi, Tokyo, Japan). This was also used to follow the hydrolytic degradation of the microspheres in vitro. The microspheres were suspended in 1 ml of phosphate buffer solution and sampled at 7, 14, and 30 days after incubation. The sampled microspheres underwent lyophilization and were coated with platinum before SEM observation.

Release of 5-FU In Vitro

Ten milligrams of microspheres, corresponding to 1 mg of 5-FU, was suspended in 1 ml of phosphate buffer solution (pH 7.4). Three different polymers were studied as the matrix of the microspheres: (1) poly(lactic acid) with a molecular weight (MW) of 4700, (2) poly(lactic acid) with a MW of 3400, and (3) 3:7 poly(glycolic-lactic acid) with a MW of 3300. The suspension was stored in a vial, and the vials were immersed in a shaker bath kept at 37°C. Approximately 1 ml of the release medium was removed periodically, and the same amount of fresh medium was added to the vial. The amount of 5-FU released into the medium was measured by bioassay using Micrococcus lutea (ATCC 10240). Drug release was studied for 7 days after incubation. The pH of the medium was measured to evaluate the effect of lactic acid or glycolic acid produced by hydrolysis.

Kinetics of Microspheres in the Vitreous

The microspheres were administered into the vitreous cavity of rabbits to study the kinetics in the eye and the tolerance of ocular tissues. To eliminate the effect of the drug, poly(lactic acid) microspheres that did not contain 5-FU were prepared in the manner described and used in the following experiments.

Ten eyes of ten pigmented rabbits, weighing 1.5–2 kg each, were used. All animals were handled according to the ARVO Resolution on the Use of Animals in Research. The rabbits were anesthetized with intra-peritoneal injection of sodium pentobarbital (20 mg/kg) before the procedures. The pupils were dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride eyedrops. The ocular surface was then anesthetized with a topical instillation of 0.4% oxybuprocaine hydrochloride.

On five of ten rabbits, 0.1 ml of a 5% microsphere suspension was injected into the vitreous cavity of the right eye through the sclera 2 mm posterior to the limbus using a 26-gauge needle. Intravitreal injection was done with observation of the fundus under an operating microscope to avoid injuries to the retina or lens. An anterior chamber paracentesis was done before the intravitreal injection to release the aqueous humor and prevent a rise in intraocular pressure. The other five rabbits received pars plana vitrectomy followed by intravitreal administration of the microspheres to the right eye. A subtotal vitrectomy was done with the three-port system (EMPAC; Optical Micro System, West Peabody, MA). The infusion needle was placed through the sclera 2 mm from the limbus in the inferior temporal quadrant, and balanced salt solution (Opegurd; Senju, Osaka, Japan) was used as an irrigating solution during the vitrectomy. After the core vitreous was removed, 0.1 ml of a 5% microsphere suspension was injected into the midvitreous cavity with a 26-gauge needle. Rabbis were excluded from the study if intraoperative complications occurred during the vitrectomy. Microspheres in the vitreous cavity were followed by fundus examination with an indirect ophthalmoscope. The left eyes of all rabbits did not receive the microsphere injection and served as controls.

Electroretinograms (ERGs) were recorded in all rabbits before and 4 weeks after administration of the microspheres. They were also recorded on the fellow control eyes. We used a jet electrode (Universe, La Chausée-Fonds, Switzerland) after 1 hr of dark adaptation. The silver plate electrodes were placed as a reference on the ear lobe and as a ground electrode on the other ear lobe. A photostimulator (Model 3G22; Nihondenki-Saney, Tokyo, Japan) with the setting of 100 μsec, 20 J, and at the distance of 15 cm from the cornea elicited a scotopic ERG. The responses were amplified with a time constant of 0.3 sec and a high cut of 1 kHz (Biological Amplifier 117B and 1243; Nihondenki-Saney). After ERG measurement, the rabbits received a fatal overdose of pentobarbital sodium, and both of their eyes were enucleated. The eyes were fixed in 10% formaldehyde-glutaraldehyde solution, dehydrated in a graded series of alcohol, embedded in paraffin, and sectioned with a microtome. The sections were stained with hematoxylin and eosin and examined under a light microscope.

Results

Characterization of Microspheres

Electron micrographs of the poly(lactic acid) microspheres are shown in Figure 2. The size of the micro-
spheres was approximately 50 μm in diameter. Also shown are the morphologic changes of the microspheres accompanying the in vitro hydrolytic degradation. The SEM observation revealed that the surface of the microspheres became irregular and porous during the degradation. It was noticed after 2 weeks that some microspheres had broken down to small fragments.

Release of 5-FU In Vitro

The release profiles of 5-FU from the microspheres with the different polymers are shown in Figure 3. From the poly(lactic acid) microspheres with a MW of 4700, 70% of 5-FU was released over 7 days. The poly(lactic acid) microspheres with a MW of 3400 released 85% of 5-FU over 7 days. Most (98%) of the drug was released from the 3:7 poly(glycolic-lactic acid) microspheres in 2 days. The profile of the drug release was accelerated by using the polymers with a smaller molecular weight or changing the polymer matrix from homopolymer to copolymer. No change in pH of the medium of phosphate buffer solution was detected after hydrolysis of the polymer microspheres into monomers of lactic acid and glycolic acid.

Kinetics of Microspheres in the Vitreous

Poly(lactic acid) microspheres were observed as a white mass in the vitreous cavity after the injection (Fig. 4A). The mass of the microspheres gradually became smaller and finally disappeared from the vitreous cavity (Fig. 4B). In eyes that did not undergo vitrectomy, the microspheres were cleared completely by 48 ± 5.2 days on average (range, 43–53 days). Clearance of the microspheres was accelerated in the vitrectomized eyes (mean, 14 ± 2.4 days; range, 11–16 days). The difference was significant (P < 0.001). No inflammatory finding was detected in the anterior chamber, lens, vitreous, or retina by slit-lamp biomicroscopy.

There were no significant changes in amplitudes of ERG b-wave before and 4 weeks after the microsphere injection (Table 1). Also, no changes in the implicit time of the b-wave were found after the injection. The eyes that received the microspheres showed no significant difference in the results of ERG examination compared with their fellow eyes, which did not receive the microspheres. Histologically, neither the
eyes receiving injection of the microspheres nor the control eyes showed any abnormality in the cornea, lens, retina, or choroid.

**Discussion**

We showed that the microspheres of biodegradable polymers are promising as a controlled-release drug-delivery system in the vitreous. Poly(lactic acid), poly(glycolic acid), and their copolymers, which are biocompatible and biodegradable, have been studied for the controlled release of many drugs, including narcotic antagonists, corticosteroids, local anesthetics, luteinizing hormone releasing hormone, and anticancer agents. The drugs are incorporated physically in the polymer matrix of the microspheres. The drug is released by diffusion through the matrix of polymers, and degradation of the matrix occurs simultaneously. In this study, the rate of 5-FU release from the microspheres was controllable by changing a molecular weight of the polymers or by using copolymer; this was consistent with previous studies. The drug was released from the microspheres over 2–7 days. We previously reported that aclarubicin hydrochloride, one of the potent anticancer agents, could be released over a 30-day period from poly(lactic acid) microspheres. We speculated that the difference in the release period of these two drugs was due to the lipid solubility of the drugs. Lipophilic drugs can distribute more homogeneously in the matrix of poly(lactic acid), which is also lipophilic, and can be released for a longer time. We incorporated 5-FU into the microspheres because the drug has been studied widely and shown to inhibit cellular proliferation in the vitreous and in the episcleral tissues after glaucoma filtering surgery. Our results showed that the microspheres with 5-FU could be prepared to release the drug for up to 7 days. We are now studying microspheres containing lipophilic antimetabolic drugs to extend the period of drug release.

Our study also showed that administration of the microspheres into the vitreous had no adverse effects on the ocular tissues. The polymers of the microspheres undergo hydrolysis in the eye, yielding monomers of lactic acid and glycolic acid. These monomers could be metabolized and removed from the eye. The microspheres were cleared completely from the vitreous cavity by 48 days on average. Clearance was accelerated from the vitreous cavity after vitrectomy (14 days). Degradation of the microspheres by hydrolysis takes about 30 days in phosphate buffer solution in vitro. Previous studies suggest that substances diffuse

**Table 1. ERG parameters in the microsphere-injected eyes and controls**

<table>
<thead>
<tr>
<th>Eyes (n = 10)</th>
<th>Scotopic B-wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (μV)</td>
<td>Implicit time (ms)</td>
</tr>
<tr>
<td>Controls</td>
<td>440 ± 40</td>
</tr>
<tr>
<td>Before microsphere injection</td>
<td>410 ± 72</td>
</tr>
<tr>
<td>Four weeks after microsphere injection</td>
<td>390 ± 62</td>
</tr>
</tbody>
</table>

* There were no statistically significant differences between controls and the microsphere-injected eyes, before or after injection of the microspheres. The values are mean ± SD.
faster in pathologic vitreous and that clearance of the drug from the vitrectomized eyes is faster than from the normal vitreous. Accelerated clearance of the microspheres from the vitrectomized eyes may be due to facilitated diffusion in the vitreous cavity. Electrophysiologic and histologic studies revealed that the microspheres and their degradation products were not toxic to the retina and other ocular tissues. These polymers, used for absorbable surgical sutures since 1975, are biocompatible. Olsen et al recently studied possible use of biodegradable polymers as a device for mechanical retinal fixation in retinal detachment surgery.

Liposomes also were studied as a slow-releasing drug-delivery system in the vitreous. Experimental and preliminary clinical studies suggest an advantage of liposomes for treatment of chronic vitreoretinal diseases. A drug-delivery system with liposomes has also been studied as preparations for instillation or administration in subconjunctival tissues. Compared with liposomes, the microspheres of polymers provide a more controllable drug release as described. Additional advantages of the microspheres include stability of the preparation and low cost of assembly.

In conclusion, our results suggest that the microspheres of biodegradable polymers could be used as a carrier for controlled drug release in the vitreous. Further investigation of the microspheres of polymers as a drug-delivery system may lead to a new treatment for some vitreoretinal disorders.

Key words: microspheres, biodegradable polymers, drug delivery, intravitreal injection, 5-fluorouracil

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References


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